MEDICINE

Restored Heart

Results from the first transplants using stem cells in Brazil usher in the prospect of using this technique against cardiac arrest, one of the main causes of death in the world

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The target of severe criticism and recent heated discussions, a special type of cell comes back onto the scene, but this time bringing good news. These are the stem cells, intensely studied over the last five years because of their fantastic peculiarity: that of multiplying themselves and ending up as the cells of the different body tissues, as distinct as those of the skin, muscle and the nervous system. In Brazil, results from at least three research groups have come out which, in parallel with European and North American teams, consolidate the stem cells as an option for – if not a cure – at least improving the quality of life of people with serious heart problems, against which medicines no longer produce the desired effects.

Using distinct techniques, researchers from the States of Rio de Janeiro, Bahia and São Paulo have concluded that the transplanting of stem cells is a promising alternative against chronic cardiac insufficiency brought on by hypertension, obstruction of the coronary arteries and by Chagas's disease. A problem in which the heart progressively loses its capacity to pump blood, cardiac insufficiency hits between 3% and 6% of the world's population, and in Brazil between five to ten million people. The team from the Federal University of Rio de Janeiro (UFRJ) and the Pro-Cardiac Hospital, which published the most advanced data, recently obtained an international triumph for their results which they arrived at after two year's work, a short period of time when dealing with a new area worldwide. On the 13th of May, the magazine *Circulation*, the most important scientific magazine dealing with clinical cardiology, published an article in which the researchers from Rio describe the first transplants using stem cells in sufferers from chronic cardiac insufficiency. Of the fourteen patients treated, twelve are doing well and two have died, apparently through causes not linked to the use of the stem cells, according to the doctors.

"The project only went so quickly because we had already had at the UFRJ a model for transplant in rats when we initiated the study", recognizes the cardiologist Hans Dohmann, from the Pro-Cardiac Hospital. "The impression is that the totipotent use substitute the fibrous tissue for muscular cells", comments Antonio Carlos Campos de Carvalho from

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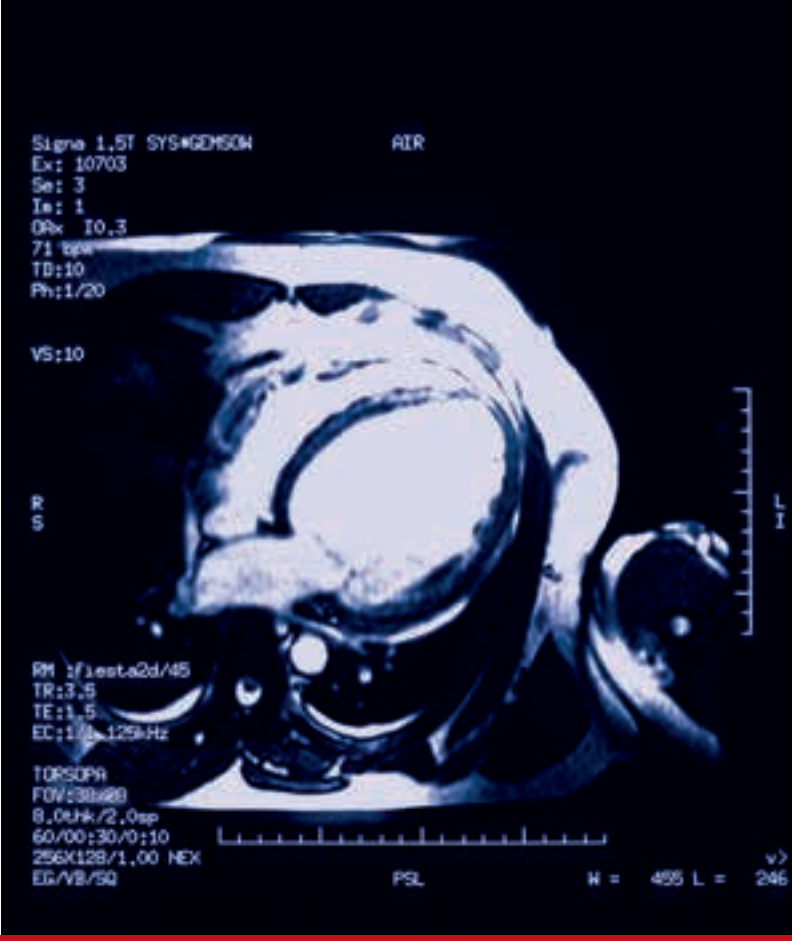
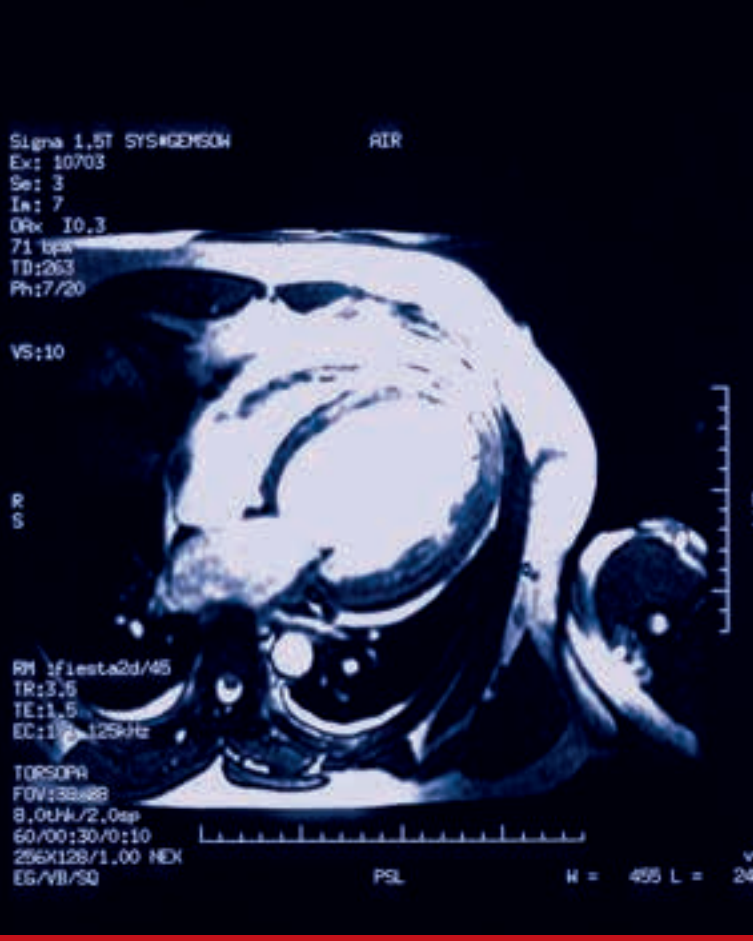
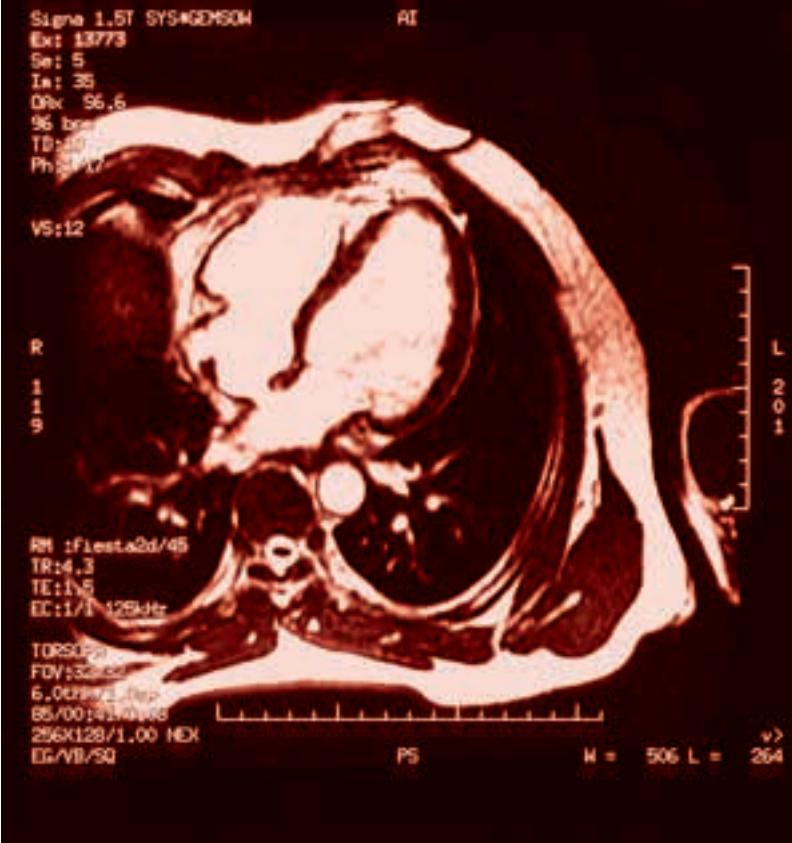




Cardiac insufficiency: the left ventricle of the heart dilates on receiving oxygenated blood from the lungs (*above*) and cannot manage to contract and pump the blood to the body

Normal heart: with thicker and more flexible walls, the left ventricle dilates on receiving the blood from the lungs and then contracts, sending the oxygenated blood to the tissues



UFRJ, which works with the Millennium Institute of Tissue Bioengineering, supported by the federal government. This treatment increases the irrigation of the injured part of the heart, allowing the cells that have entered into a type hibernation to return to contracting themselves.

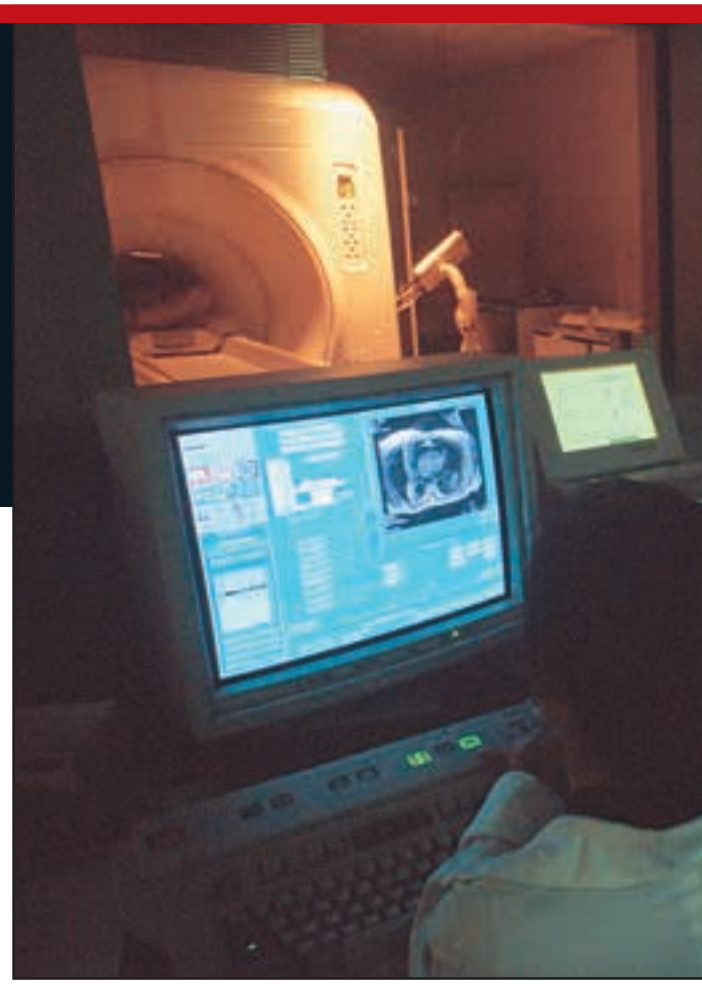
At the same time a group from the Heart Institute (Incor) and the School of Medicine of the University of São Paulo (USP) applied these cells in nine patients suffering from cardiac insufficiency caused by hypertension, Chagas's disease or of unknown origin. Two distinct techniques were used. One, by applications either of stem cells filtered from the blood of the patient himself, the other by using a hormone that stimulates the liberation of stem cells from the bone marrow to the bloodstream. Four months afterwards three were no longer on the list for heart transplants, five improved considerably and one died, death being attributed to the serious state of health the patient before entering into the experiment. "Looking at the as yet restricted number of patients, it is early to talk confidently about the efficiency of these techniques", comments the cardiologist Edimar Bocchi from USP, one of the research coordinators. "But the results point to the prospect of improvement for these people who have an extremely serious illness."

In practical terms, the teams from Rio and São Paulo have maintained beating – with a good part of their previous vigor – the hearts of men and women who could no longer walk in the morning to the baker's shop nor feed themselves, such was the dimension of the tiredness brought on by cardiac insufficiency. Before the treatment with stem cells, the only solution for them was to wait for months for a heart transplant. If they managed to resist, they would confront surgery that begins with a cut of some thirty centimeters in the thorax, finishes some nine hours later, demands a month for recovery, and in total the bill runs up to R$ 200,000. The implanting of stem cells is much simpler. Carried out by the introduction of a catheter into the artery that runs from the thigh to the heart, the operation lasts a little more than an hour, demands only two days in hospital and costs ten times less.

Such is the confidence today deposited in the use of stem cells that the National Commission of Ethics in Research (Conep in the Portuguese acronym), an organ of the Ministry of Health that authorizes medical research with human beings, approved in March of this year the proposal presented one year ago by the medical doctor Ricardo Ribeiro dos Santos, the coordinator at the Millennium Institute of Tissue Bioengineering and researcher with the Osvaldo Cruz Foundation (Fiocruz) in Salvador, Bahia. During this month, Santos in partnership with cardiologists from the Santa Izabel Hospital, also in the Salvador, intends to begin a study with five patients suffering from cardiac insufficiency caused by Chagas's disease, the illness brought on by the protozoa *Trypanosoma cruzi*, the parasite that lodges in the cells of the heart. In recent research, Santos demonstrated that the use of this type of cell reduces in a long-standing manner the inflamed and damaged areas of the heart of mice with Chagas's disease. This could be a way of keeping the healthy heart free of trouble so that under these circumstances the parasite, which causes an illness that affects sixteen million people in Latin America of who six million are Brazilians, can then be fought against.

Only at the beginning - Even with these results some years will still be necessary before this type of treatment becomes available to the population both in the public and private health systems. The experiments are merely the start of a long trajectory until the approval of new medicines or medical procedures for human beings. This is called phase 1 of clinical studies the objective of which is to investigate if the treatment is safe and does not cause any serious side effects. This is followed by other stages in which the efficiency of the treatment in tens and then thousands of patients are analyzed.

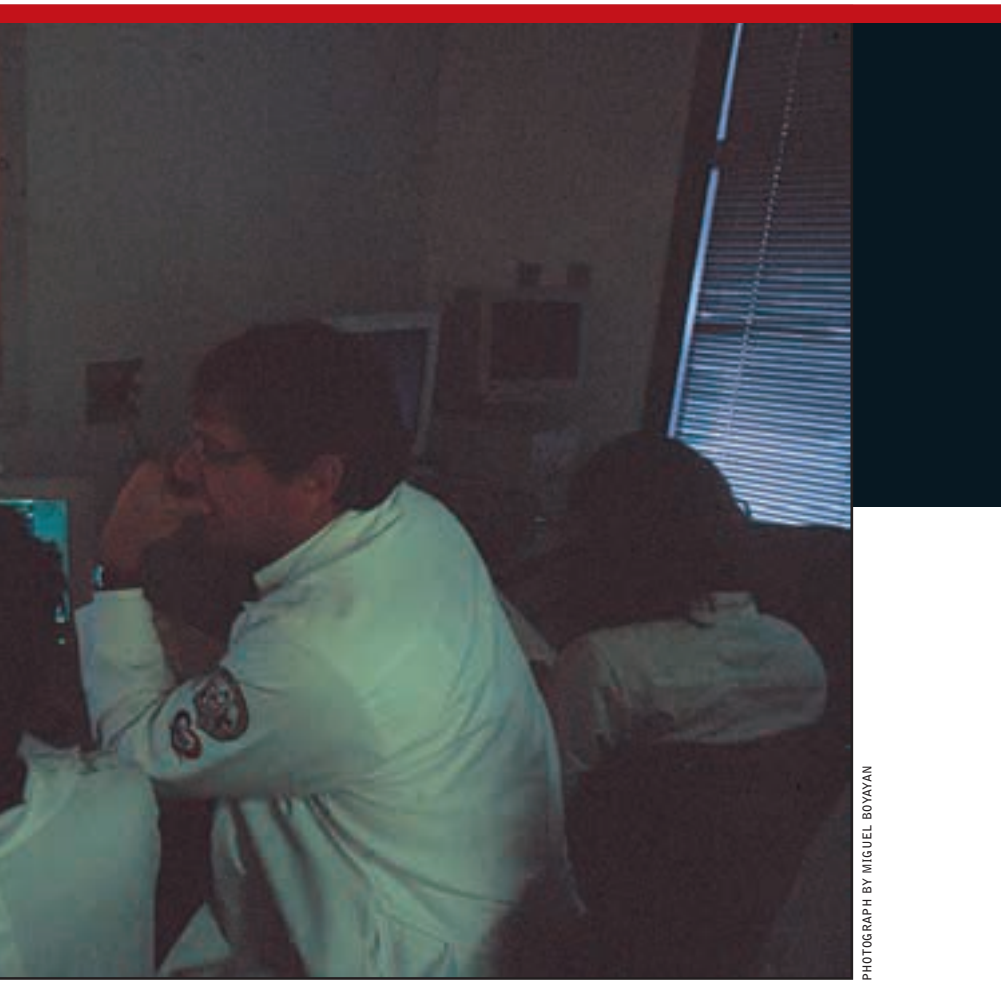
The Brazilian researchers are by-passing the ethical questions associated with the use of these cells because they are



Before and after transplant with the stem cells: the Incor team evaluates via nuclear magnetic resonance (NMR) the capacity of the heart to pump blood

working with only one of the two types in existence. They are using adult stem cells, produced by the bone marrow of the individual himself that he will later receive in the transplant. In this manner, they are avoiding the controversial question surrounding the use of the other type of stem cells, the embryonic stem cells, so-called because they are removed from embryos with only a few days of life. This is exactly the reason for the controversy as the embryo dies when the cells are extracted. More versatile than the adult cells, the embryonic stem cells are capable of originating all types of body cells. For this reason, countries with legislation considered to be more liberal, such as Britain, have limited research to the cells removed from embryos discarded in the treatment of assisted fertilization. Nobody can also forget the more conservative attitude adopted by the President of the United States, George W. Bush, who has restricted federal financing only to studies with sixty two strains of embryonic stem cells already characterized in the laboratory.

In a situation in which the scientific result receives help by accident, the project of the implanting of stem cells in the heart was born from a solution in search of a problem. During 2000, Hans Dohmann, from the Pro-Cardiac Hospital in Rio, had been working with the Brazilian cardiologist Emerson Perin at the Texas Heart Institute, building a special catheter using a fine needle and sensors capable of identifying the dead portions of the heart. In this same year, they got to know at a meeting in Hamburg, Germany, that another team had completed the catheter before them. It was at that point that the specialists recognized that the gadget could be used in research with stem cells. "On the plane coming back to Rio, I was imagining that we would need cooperation with foreign teams in the case I decided to get involved in this area", recalls Dohmann.



PHOTOGRAPH BY MIGUEL BOYAYAN

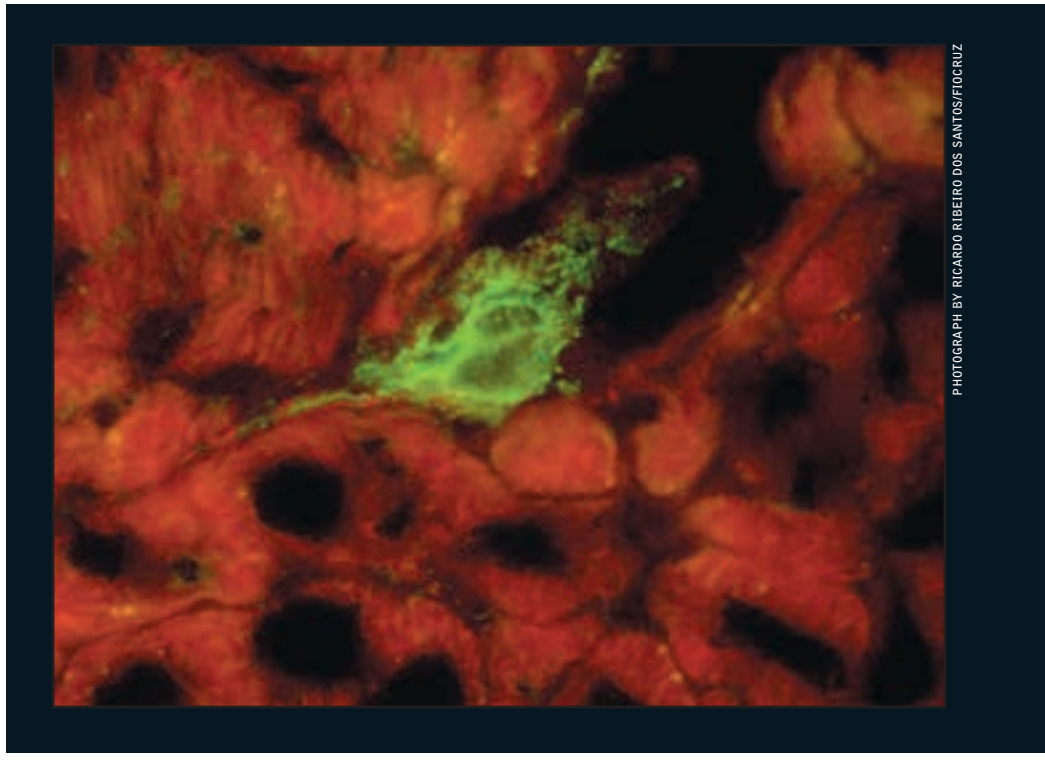
By chance he had fooled himself. In Rio, Radovan Borojevic and Antonio Carlos Campos de Carvalho, both from UFRJ, had initiated a study on the application of stem cells to mice with artificially brought on cardiac insufficiency. This is something similar to what is observed when there is an accumulation of fatty tissue in the coronary arteries that diminishes the flow of blood to the heart. This reduction kills some areas of the heart, which then stops pumping blood in an efficient manner to the body. In an attempt to compensate for the lack of force, the heart increases in size, reaching double the normal in the most serious cases, as it can be seen on page 23. The green light for the work came through in October of 2001, five months after Carvalho and Borojevic had verified in mice that the stem cells incorporated themselves into the heart muscle, and, even more importantly, restored, at least in part, its capacity to self-contract. "There are as yet no detailed studies that explain what is happening", comments Carvalho.

Little by little the researchers from Rio demonstrated, in a pioneering manner, that this type of cellular therapy re-establishes the pumping of the blood by the heart in the most complicated cases of cardiac insufficiency, in which the problem turns chronic and the patient feels fine only when he is sitting down. Under these conditions, the medicines that impede the increase of the size of the heart – such as beta-blockers and the inhibitors for the angiotensin converting enzyme – stop producing the desired effect. In some cases, it also becomes impossible to treat the disease using traditional therapies such as angioplasty, the introduction of a catheter with a balloon on the tip, which squashes the plaques of fat, or the implant of a bridge, a way of detouring the blocked area by using veins removed from the leg or the pectoral region. In the most severe cases, cardiac insufficiency kills half of the patients in six months.

Before this Brazilian study, Bodo Strauer, from the University of Düsseldorf, in Germany, had published in September of 2002 in the *Circulation* magazine an article in which he described an increase in the blood flow rate of the heart in patients who had received applications of stem cells. However, the study took into consideration only people

who had suffered acute heart attacks and where part of the heart had remained close to a week with out an adequate supply of blood. Furthermore, the capacity of these patients to pump blood was higher than the one shown by the Brazilians – which made their recovery simpler than when the problem became chronic.

With the approval of Conep, Dohmann and Perin selected their twenty-one volunteers who were integrated into the initial phase of the clinical study. Each person who was submitted to the therapy with stem cells passed through the same procedure: by way of a small cut in the highest portion of the hip, the doctors introduced a needle into the ilium bone, which forms the bulging sides of the pelvis, and they extracted 50 ml of material drawn from the bone marrow. This viscous and dark red colored liquid consists of a mixture of cells, very rich in adult stem cells that are afterwards separated in the laboratory.



PHOTOGRAPH BY RICARDO RIBEIRO DOS SANTOS/FIOCRUZ

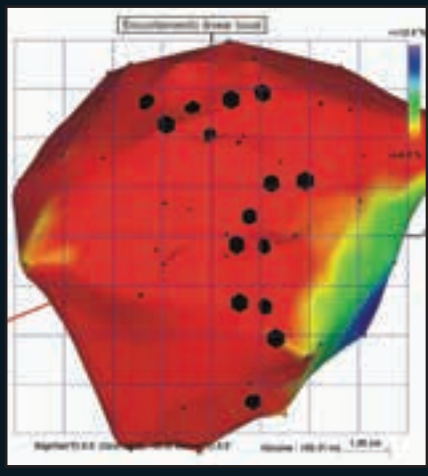
Back in the surgery theater four hours later, Dohmann and Perin had in their hands a red concentrate of live stem cells. With the catheter, thirty million of them were injected into the internal wall of the left ventricle – the most important of the four heart chambers, which pumps blood rich in oxygen to the body. They applied the stem cells in the areas in which the cardiac muscle had gone into a type of hibernation – inactive although still alive. Four months afterwards it was noted that the transplant had stimulated the appearance of small arteries in the region of the heart that had lost the capacity to self-contract.

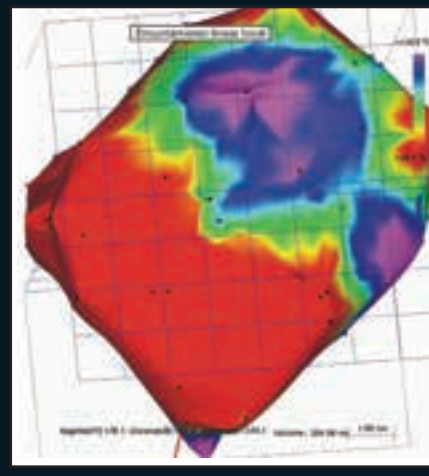
With the improvement in the blood flow rate, the area that is short of blood decreased by 73% and the capacity to pump from the heart increased from 20% to 29%, sufficient to allow that the transplanted patients increased from five to seven minutes their time for slow steps on a treadmill. "It may seem small but this allowed these people to carry out activities that had become impossible through cardiac insufficiency such as changing their own clothes", relates Dohmann.

The most exciting fact is that up until this moment the technique used on the group from Rio has not caused any serious complication, such as an alteration in heart beat (arrhythmia), which could lead to heart stoppage. The doctors have compared the quality of life of the people who received the stem cells before the treatment and six months after the application. On average, they found them the same as or better than Americans of the same age. The seven members of the control group who took only medicine against cardiac insufficiency did not present any relevant improvement in the condition of their health during the same period. According to Dohmann, they should also receive the implantation of stem cells, possibly starting in August. In a second phase of this study, forecast to begin at the end of this year, the researchers from Rio de Janeiro intend to analyze the performance of this technique in a larger group composed of one hundred and twenty patients with chronic cardiac insufficiency.

Cellular reinforcement - In São Paulo the cardiologists Edimar Bocchi and Dalton Chamone have also verified an increase in the force of the heartbeat, restored by techniques distinct from the treatment with stem cells. Instead of extracting these cells directly from the bone and injecting them into the cardiac muscle, Bocchi and Chamone were able to count upon the collaboration of the organism itself. On average, during a five-day period they applied to their patients, injections of 600 micrograms of a special protein, the granulocyte-macrophage colony-stimulating factor. (GM-CSF), which make the stem cells migrate from the bone to the bloodstream. When they reach a certain concentration the doctors sent the transplant candidates for a series of blood filtering to separate out the stem cells which were then frozen and stored before being once again injected into the bloodstream.

Of the nine patients between the ages of thirty-three and sixty-five, two received injections of stem cells into their coronary arteries by way of a catheter. The other seven only injections of GM-CSF – and all of the remaining work had to be done by the organism itself. It is believed that the damaged tissue, like that of the injured heart, exercises a type of chemical attraction on the stem cells through the release of proteins that spur the communication between cells, such as the interleukin 6 and the Human Tumor necrosis factor-alpha. It was not only the methods





IMAGES BY HANS DOHMANN/PRÓ-CARDÍACO

Results: on the left, stem cell (*in green*) lodged in the heart of a mouse with Chagas's disease. Above, map of the left ventricle with implantation points of totipotent cells and improved blood flow

that varied. The people treated at Incor had cardiac insufficiency caused not by blocking of the veins but by causes as varied as Chagas's disease, increase of blood pressure (hypertension) or even an increase in the size of the heart without any known reason – an infirmity known in medical jargon as idiopathic dilated cardiomyopathy. In a general manner, the capacity to pump blood and the oxygen intake increased and three patients had their names taken off the heart transplant list. Today they are only taking the medicine needed to control cardiac insufficiency. Two of them still present a problem that normally reaches 20 % of cardiac insufficiency sufferers: the blocking by coagulant blood of the artery that leads the blood poor in oxygen to the lungs. "An apparent small increase in the capacity to pump blood many times represents a significant improvement for the patients", Bocchi explains.

Against Chagas's disease - In Salvador, Santos from Fiocruz, and Fabio Vilas-Boas Pinto from the Santa Izabel Hospital, intend to use stem cells to specifically reverse the damage that Chagas's disease produces on the heart. After getting into the blood stream, the parasite that causes the disease, the protozoa named *Trypanosoma cruzi*, lodges in the interior of the cardiac cells, which sets off an alert to the immune system. But the parasite is not the only one that loses. The heart cells have on their surface proteins similar to those of trypanosome and for this reason they also suffer an attack from the defense cells. As a consequence, thousands of scars appear throughout the organ. Both in Chagas's disease and in a heart attack, which leads to the death of a large area of the heart, the result is similar: 30% of the sufferers from Chagas's disease acquire the illness during infancy and only around forty years of age do they develop progressive cardiac insufficiency that leads to their death in ten years.

When insufficiency gets worse, the only way out is a heart transplant, not very efficient since the protozoa that remain in the bloodstream infect the recently implanted organ. An aggravation to the problem is that infection by the trypanosome is more frequent in the population of rural areas, especially in the North and Northeast Regions in which there are no heart transplant programs. Stem cells could alleviate this problem. In mice, as Santos verified, one only needs to give an injection of 20 million stems cells associated to the use of the granulocyte-macrophage colony-stimulating factor, the GM-CSF, in order to reduce the inflammation and the fibrous tissue of the heart. After two months, the mice treated using this method showed 80% fewer inflamed cells and fibrous tissue than the mice that had not received this therapy. The most important aspect is that this benefit seems to be long lasting: the improvement persisted for six months after the mice received the stem cells, a period equivalent to almost twenty years for human beings.

In a recently approved experiment by Conep, Santos is going to inject 30 million of these cells into the interior of the coronary arteries, as well as GM-CSF, initially in five patients with cardiac insufficiency caused by Chagas's disease. If the technique shows itself to be secure, the researcher from Fiocruz will widen and detail the study with a further twenty-five patients – five should receive stem cells and GM-CSF, ten will be treated only with stem cells and ten with medicine. "Using this therapy we hope to reduce the lesions on the heart to the minimal level, similar to those that occur with 70% of the carriers of Chagas's disease who do not develop cardiac insufficiency", says Santos. If a success, the treatment will allow that the doctors can give to the patients a medicine to combat the protozoa, named benznidazole, which, as it is toxic, cannot be used with people suffering from cardiac insufficiency. •

THE PROJECT

Cellular Therapies for Degenerative Chronic Illnesses

COORDINATOR
Ricardo Ribeiro dos Santos – Millennium Institute of Tissue Bioengineering

INVESTMENT
R$ 5,200,000.00 (Ministry of Science and Technology), R$ 500,000.00 (Pro-Cardiac Hospital), R$ 500,000.00 (Fiocruz-BA), R$ 200,000.00 (Faperj)